Reference: *Biol. Bull.*, **141**: 162–166. (August, 1971)

LARVAL DEVELOPMENT OF *PAGURUS LONGICARPUS* SAY REARED IN THE LABORATORY. IV. ASPECTS OF THE ECOLOGY OF THE MEGALOPA[1]

MORRIS H. ROBERTS, JR.[2]

Virginia Institute of Marine Science, Gloucester Point, Virginia 23062

Many animals are able to delay metamorphosis to the juvenile or adult stage until some stimulus has been received from a suitable substrate (Wilson, 1952, 1958; Crisp and Barnes, 1954; Scheltema, 1961; and many others). In some cases the physiological basis for delayed metamorphosis has been carefully investigated (for example, Meadows, 1964a, 1964b, 1964c).

During or immediately after the megalopal instar, the hermit crab must find a suitable shell in which to house its abdomen in order to protect itself from predation. For oceanic species, delayed metamorphosis might be an adaptation to permit larvae to reach the bottom while still capable of locomotion in the pelagic environment (Bouvier, 1905). Thompson (1903) suggested that lack of a shell could delay metamorphosis. Thompson (1903) and Bookhout (1964) have both reported that megalopae without shells have a higher per cent mortality than those with shells. If this is true, it implies physiological stress resulting from lack of a shell as does the observation that shell-less adults do not feed (Allee and Douglis, 1945).

None of these hypotheses has been rigorously tested in controlled experiments. Thompson (1903) tested the effect of various shells on megalopae, but his conclusions are questionable (see below). Reese (1962, 1968) and Hazlett and Provenzano (1965) have studied the behavior associated with selection and entry of shells by megalopae. Reese (1968) has made some observations on the role of shells in emigration of *Birgus latro* onto land and metamorphosis to the juvenile.

The present study was made specifically to test the effect of shell presence on mortality and intermolt duration of the megalopa of *Pagurus longicarpus*.

Materials and Methods

Megalopae used in these experiments were obtained from a number of different cultures established for various experiments on zoeal instars. Culture conditions up to the megalopa differed between experiments, but were the same within each given experiment. In all experiments, the salinity was maintained as it was during zoeal development (23.7‰ in Experiment 1, 30.0‰ in Experiment 2, 25.5‰ in Experiment 3). Temperature was quite variable as culture dishes were kept on a sea table. The mean temperature was 24° C (21–26° C) in Experiment 1, 24.5° C (19–27° C) in Experiment 2, and 22° C (18–24° C) in Experiment 3. Megalopae were transferred to freshly prepared environments on a variable schedule. Mortality, molting, and shell entry were noted daily. The shells

[1] Contribution Number 387 from the Virginia Institute of Marine Science, Gloucester Point, Virginia 23062.

[2] Present address: Department of Biology, Providence College, Providence, Rhode Island 02918. This paper is part of a dissertation submitted to the School of Marine Science of the College of William and Mary in partial fulfillment of the requirements for the Doctor of Philosophy Degree.

in every experiment were *Bittium*, cleaned of organic matter with KOH. These proved very suitable in size and were easily collected from *Zostera* beds where they abound on nearly every blade.

In Experiment 1, three variables were involved: sand substrate, shell, and nauplii of *Artemia*. Eighteen megalopae were maintained individually in compartmented plastic boxes in each of six different environments, making a total of 108 megalopae for the entire experiment. The six environments were: no sand substrate, no shell, *Artemia;* no sand substrate, shell, *Artemia;* sand substrate, no shell, *Artemia;* sand substrate, shell, *Artemia;* sand substrate, no shell, no *Artemia*, sand substrate, shell, no *Artemia*.

In Experiments 2 and 3 the design was greatly simplified with only a single variable, shell presence or absence. No sand substrate was provided; nauplii of *Artemia* were added as food. In Experiment 2, 62 megalopae were presented a shell, 61 were not, for a total of 123 megalopae. In Experiment 3, 115 megalopae were presented a shell, 113 were not, for a total of 228. These experiments differed in the salinity-temperature regime to which the megalopae were subjected and hence are not exactly comparable.

Results

Mortality

In Experiment 1, no significant difference in numbers alive and dead was detected at the 95% confidence level among the various treatments when tested by an R × C contingency test, indicating that there was no effect of any treatment on mortality. Per cent mortality for megalopae with shells was 16.7% compared to 13.0% for those without shells, 14.8% overall. Some deaths are known to have resulted from predation by small *Nereis* sp. introduced with the sand substrate (about 5.6% for the four environments with sand substrate). No correction of per cent mortality is possible, however, since it is not known whether the *Nereis* were distributed randomly with and without shells. I believe that there was a greater proportion of *Nereis* with megalopae having a shell.

In Experiments 2 and 3, per cent mortality was slightly greater for megalopae without shells (27.9 and 26.5%, respectively) than for those with shells (17.8 and 19.1%, respectively). In each case no significant difference in the numbers alive and dead under each treatment was detected when tested by an R × C contingency test. Although these two experiments are not directly comparable because of different sources of larvae and temperature-salinity regime, the overall mortality for both was 22.8%. This is markedly higher than in Experiment 1 and the salinity tolerance experiment described elsewhere (Roberts, 1971). This is attributed to the fact that larvae in Experiments 2 and 3 were not culled for the most active and healthy megalopae whereas in the other experiments only active megalopae were used.

Shell entry

The behavioral patterns of larvae entering a shell have already been described by Reese (1962) for laboratory reared megalopae of *P. longicarpus*, and by Hazlett and Provenzano (1965) for several other species, reared or collected from the plankton. Nothing can be added to their observations in this regard.

Shell entry can occur at any time during the megalopal instar. Of the megalopae which successfully entered shells, 46 to 50% did so within 24 hours and 82 to 93% within 48 hours. In each experiment, some larvae failed to enter the shell provided during the megalopal instar; 18.8% in Experiment 1, 21.6% in Experiment 2, but only 1% in Experiment 3. The disparity in this respect cannot be explained. These larvae did enter a shell immediately after molting to the first juvenile instar. Individuals deprived of a shell during the megalopal instar were given shells immediately after ecdysis and required only 5 minutes or less to gain entry.

Intermolt duration

Intermolt duration for megalopae with and without shells was compared for each experiment. For Experiment 1, no significant difference was found in the response of larvae to the various treatments in a preliminary R × C contingency test. The data were then pooled according to presence and absence of shell. Mean intermolt durations were 3.6 and 3.8 days for megalopae with and without shells, respectively. No correction was made for those larvae that failed to enter a shell during the test period.

In Experiment 2, correction was made for crabs that failed to enter a shell during the megalopal instar, these being added to the group not presented shells. The mean intermolt durations were 4.3 and 4.4 days for megalopae with and without shells, respectively.

Again in Experiment 3, there was no significant difference in intermolt duration for megalopae with and without shells. Mean intermolt duration was 4.9 days for megalopae with shells, 5.0 days for megalopae without shells. In this experiment, with only 1% of the larvae failing to enter a shell, no correction was made for these larvae.

Discussion

Thompson (1903), testing the effect of various shells on megalopae of *Pagurus annulipes,* reported high mortality, ranging from 30 to 60% (excluding a group of 10 larvae in Series B_1). Megalopae without shells experienced 50% mortality (Thompson's stated value of 81% on page 186 seems in error), while megalopae with shells experienced only 44% mortality. In some experiments with a 3 or 4 day delay in presentation of the shell, the per cent mortality was even lower, rather than between 44 and 59% as would be expected. Nevertheless, Thompson concluded that megalopae without shells have a higher mortality than those with shells. Bookhout (1964) reached the same conclusion in his experiments with *Pagurus bernhardus.* In his experiments, larvae without shells experienced about 64% mortality (7 of 11) while mortality for larvae with shells was only about 7% (1 of 14).

In my experiments, mortality was unaffected by shell presence or absence. The discrepancy between the results of Thompson (1903), Bookhout (1964) and myself arises from the fact that in the experiments of the first two investigators megalopae were not isolated, whereas in my experiments they were. It has frequently been noted that megalopae in mass cultures inflict serious, often fatal wounds on one another. The difference in mortality for megalopae with and without shells in the experiments of Thompson (1903) and Bookhout (1964)

reflects the role of shells in protecting the megalopae and later instars from predation, whether by members of their own species, or by other animals (as for example in Experiment 1).

The megalopa is initially an active swimmer, using its setose pleopods to propel itself, anterior end foremost, with the thoracic appendages held close to the carapace and with dactyli pointed forward much as in the brachyurans (Atkins, 1954). Swimming activity, though not quantified, definitely declined with time as was previously noted for *Birgus latro* (Reese, 1968). If a megalopa encounters a shell, it enters and swimming behavior ceases immediately if the shell is suitable. If no suitable shell is located, active swimming is nevertheless very infrequent after two days. By this time, the majority of megalopae are already housed in shells. The reduction in swimming activity is correlated with reduction of the pleopod musculature preparatory for metamorphosis as described by Thompson (1903).

Bouvier (1905) observed a very wide size range for what he believed to be conspecific hermit crab megalopae from the plankton. He postulated that failure to reach the bottom caused megalopae to delay metamorphosis and instead pass through a number of megalopal instars of increasing size. His identification of the megalopae as conspecific was assuredly incorrect since the megalopae ranged from 4 to 20 mm and differed in anatomical detail. His point is well taken that failure to reach the bottom or locate a suitable substrate with shell could result in delayed metamorphosis, as has been shown in a number of other organisms (see citations in the introductory remarks); however evidence for this phenomenon is lacking for hermit crabs.

Thompson (1903) examined the effect of dextral, sinistral, and straight shells, delayed introduction of shells, and shell absence on the intermolt duration of megalopae of *P. annulipes*. Intermolt durations in his experiments ranged from 4.4 to 5.4 days. He concluded that shell absence caused a longer mean intermolt duration (5.4 days) than shell presence (4.9 days, his Series A plus controls in Series B). However, a statistical comparison of his data reveals no significant difference in intermolt duration, which agrees with the results of the present experiments. The greater variability of Thompson's results may be a function of variable temperature and, in some cases, small sample size.

Reese (1968) reported that an unhoused *Birgus latro* megalopa failed to metamorphose after some 30 days whereas housed larvae metamorphosed after 21 to 28 days. Provenzano (1962) reported a *Coenobita clypeatus* megalopa that failed to metamorphose after 31 days in water although provided with a shell. This is the instar during which these species first emigrate to land. Clearly, megalopae of the terrestrial hermit crabs belonging to the family Coenobitidae have a long intermolt duration (20 to 30 days), but it is not clear from the results of Reese (1968) and Provenzano (1962) whether metamorphosis was actually delayed, and if so, whether the delay resulted from the effect of shell absence or immersion. Further experiments are needed to elucidate this and other points concerning the ecology of the megalopa of terrestrial species.

I greatly appreciate the help and encouragement of Dr. Langley Wood, chairman of my graduate committee. Dr. Morris L. Brehmer kindly provided space in his laboratory. Dr. Marvin L. Wass and Mssrs. John J. Norcross and

Willard A. Van Engel critically reviewed the manuscript. Special thanks go to my wife, Beverly Ann, for her help in culturing many animals used in these experiments.

During the course of this study I was the recipient of a National Science Foundation Predoctoral Fellowship.

Summary

1. Mortality of isolated megalopae is unaffected by shell presence or absence. In mass culture, shell-less megalopae have a higher mortality because of cannibalism.

2. After 24 hours, 50% of the megalopae have entered a shell if available; after 48 hours, up to 93%. A few megalopae which failed to enter a shell did so immediately after the molt to the juvenile instar.

3. Intermolt duration was not significantly affected by shell presence or absence.

LITERATURE CITED

Allee, S. C., and M. B. Douglis, 1945. A dominance order in the hermit crab *Pagurus longicarpus* Say. *Ecology*, **26**: 411–312.

Atkins, D., 1954. Leg disposition in the brachyuran megalopa when swimming. *J. Mar. Biol. Ass. U. K.*, **33**: 627–636.

Bookhout, C. G., 1964. Salinity effects on the larval development of *Pagurus bernhardus* (L.) reared in the laboratory. *Ophelia*, **1**: 275–294.

Bouvier, E. L., 1905. Nouvelle observations sur les Glaucothoès. *Bull. Inst. Oceanogr. Monaco*, **2**: 1–15.

Crisp, D. J., and H. Barnes, 1954. The orientation and distribution of barnacles at settlement with particular reference to surface contour. *J. Anim. Ecol.*, **23**: 142–162.

Hazlett, B. A., and A. J. Provenzano, Jr., 1965. Development of behavior in laboratory reared hermit crabs. *Bull. Mar. Sci.*, **15**: 616–633.

Meadows, P. S., 1964a. Substrate selection by *Corophium* species: the particle size of substrates. *J. Anim. Ecol.*, **33**: 387–394.

Meadows, P. S., 1964b. Experiments on substrate selection by *Corophium* species: films and bacteria on sand particles. *J. Exp. Biol.*, **41**: 499–511.

Meadows, P. S., 1964c. Experiments on substrate selection by *Corophium volutator* Pallas: depth selection and population density. *J. Exp. Biol.*, **41**: 677–687.

Provenzano, A. J., Jr., 1962. The larval development of the tropical land hermit *Coenobita clypeatus* (Herbst) in the laboratory. *Crustaceana*, **4**: 207–228.

Reese, E. S., 1962. Shell selection behavior of hermit crabs, *Anim. Behav.*, **10**: 347–360.

Reese, E. S., 1968. Shell use; an adaptation for emigration from the sea by the coconut crab. *Science*, **161**: 385–386.

Roberts, M. H., Jr., 1971. Larval development of *Pagurus longicarpus* Say reared in the laboratory. II. Effects of reduced salinity on larval development. *Biol. Bull.*, **140**: 104–116.

Scheltema, R. S., 1961. Metamorphosis of the veliger larvae of *Nassarius obsoletus* (Gastropoda) in response to bottom sediment. *Biol. Bull.*, **120**: 92–109.

Thompson, M. T., 1903. The metamorphosis of the hermit crab. *Proc. Boston Soc. Natur. Hist.*, **31**: 147–209.

Wilson, D. P., 1952. The influence of the nature of the substratum on the metamorphosis of the larvae of marine animals, especially the larvae of *Ophelia bicornis* Savigny. *Inst. Oceanogr., Monaco, Ann.*, **27**: 49–156.

Wilson, D. P., 1958. Some problems in larval ecology related to localized distribution of bottom animals. Page 87 in A. Bussati-Traverso, Ed., *Perspectives in Marine Biology*. University of California Press, Berkeley, California.